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Vol. 85 August 1998 No. 2

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DNA GENETIC DIVERSITY OF *VETIVERIA ZIZANIOIDES* (POACEAE)

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ABSTRACT

Seventeen world wide accessions of *Vetiveria zizanioides* and six accessions of putative *V. nemoralis* from Thailand were analyzed by the use of random amplified polymorphic DNAs (RAPDs). A vetiver accession from Panamá was found to be the most distinct. All of the putative *V. nemoralis* from Thailand clustered together and the remaining *V. zizanioides* accessions formed a tightly clustered group. Within *V. zizanioides*, several accessions were found that differ from the commonly utilized "Sunshine" cultivar. These new vetiver germplasm sources will be evaluated in replicated test plots and potentially new lines of germplasm can be utilized for soil erosion control in the tropics.

KEY WORDS: geographic variation, vetiver, *Vetiveria nemoralis, V. zizanioides*, Poaceae, random amplified polymorphic DNAs, RAPDs

Vetiver (*Vetiveria zizanioides* [L.] Nash) has been utilized in many parts of the world to control soil erosion. Hedges of the non-seeding vetiver provide an effective living dam against erosion (NRC 1993) and this technique is now in use in more than 100 countries. The origin of the non-seeding vetiver is not known. However, *V. zizanioides* seems to have originated in the area from India to Vietnam and its fragrant roots have been used for centuries for mats and perfumes (NRC 1993).

Kresovich *et al.* (1994) reported on clonal variation of vetiver using random amplified polymorphic DNAs (RAPDs). They found RAPD patterns were very stable within clones and were able to distinguish between various vetiver accessions. Adams & Dafforn (1998) examined 121 accessions of vetiver and found that 86% of these appeared to be from a single clone (no variation in the DNA examined). This work was expanded in Adams *et al.* (1998) to include closely related genera (*Chrysopogon* and *Sorghum*). Since these studies, we have found several other genotypes of vetiver and in the present paper we compare these genotypes with six putative *V. nemoralis* Camus accessions from Thailand.

MATERIALS AND METHODS

Specimens were collected as given in Table 1. The leaf samples were shipped desiccated in silica gel (Adams *et al.* 1992). The DNA from vetiver is not preserved well in either fresh or air dried leaves. Interim preservation of the leaves in silica gel is necessary. Upon receipt, all the materials were frozen until analyzed. DNA was extracted using the hot CTAB protocol (Doyle & Doyle 1987) with the addition of 1 % (w/v) PVP and Proteinase (150 μg). Grinding in hot CTAB (60° C) in a hot mortar and pestle was somewhat effective for some accessions, but most accessions yielded larger molecular weight DNA and greater yields when the tissue was ground in liquid nitrogen and then placed in hot CTAB.

PCR was performed in a volume of 15 μl containing 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.0 mM $MgCl_2$, 0.01% gelatin and 0.1% Triton X-100, 0.2 mM of each dNTPs, 0.36 μM primers, 0.3 ng genomic DNA, and 0.6 unit of Taq DNA polymerase (Promega). A control PCR tube containing all components, but no genomic DNA, was run with each primer to check for contamination.

The following fifteen, ten-mer primers (numbers from U. British Columbia project) were used: 131, GAA ACA GCG T; 134, AAC ACA CGA G; 153, GAG TCA CGA G; 184, CAA ACG GAC C; 212, GCT GCG TGA C; 218, CTC AGC CCA G; 234, TCC ACG GAC G; 239, CTG AAG CGG A; 249, GCA TCT ACC G; 250, CGA CAG TCC C; 265, CAG CTG TTC A; 268, AGG CCG CTT A; 327, ATA CGG CGT C; 338, CTG TGG CGG T; 347, TTG CTT GGC G. These primers gave several bright bands, did not have any false bands (in the controls) and were proved to be reproducible in replicated analyses. DNA amplification was performed in an MJ Programmable Thermal Cycler (MJ Research, Inc.). The thermal cycle was: 94° C (1.5 min) for initial strand separation, then 40 cycles of 38° C (2 min), 72° C (2 min), 91° C (1 min). Two additional steps were used: 38° C (2 min) and 72° C (5 min) for final extension. Amplification products were analyzed by electrophoresis on 1.5% agarose gels and detected by staining with ethidium bromide. The gels were photographed under UV light with Polaroid film 667. pGEM DNA (Promega) was used as a molecular weight marker. The RAPD bands were scored by molecular weight and assigned a code based on primer number prefix and molecular weight category. In addition, the RAPD band intensity was scored as: 0 = no band; 4 = faint; 5 = medium; 6 = bright band, in reference to a gray tone standard (Adams & Demeke 1993). It might be noted that analyses using simple 0 = absent and 1 = present scoring gave very similar results, except the eigenroots were not as strongly loaded on the first few axes, implying that the information content was less than when the 0 - 6 scale was used. In replicated analysis, we have found that the relative band intensity is very reproducible in our lab. In our RAPD analyses, every primer generated at least one very bright band (level 6). Over the past several years we have screened over 250 primers and selected about fifteen primers that we use routinely. Any primer that did not generate at least one level 6 band (very bright) was not used in the analyses. In addition, if the PCR amplification does not result in at least one level 6 band, the sample is re-analyzed in triplicate. Invariably, upon re-amplification all three re-analyses resulted in at least one level 6 band. The brightest of the triplicate samples is then re-electrophoresed with the other samples. This iterative approach results in obtaining a set of very similar amplifications for each sample. Thus, the relative intensities are preserved.

Several factors may be responsible for the presence of faint bands: single copy DNA for faint bands vs. multiple DNA copies for bright bands; tertiary folding of DNA with cross bonding making the DNA less amenable to PCR amplification; and competitive interactions between bands for TAQ enzymes and substrates during amplification.

These data were coded into a matrix of taxa by character values. Similarity measures were computed using absolute character state differences (Manhattan metric), divided by the maximum observed value for that character over all taxa (= Gower metric; Gower 1971; Adams 1975). Division by the character state range was tried and found to be less informative than using the maximum observed character value (*i.e.*, including zero in the range). Principal coordinate analysis (PCO) of the similarity matrix follows Gower (1966) by program PCO3D (available for PC computers from RPA).

Table 1. Vetiver accessions used in this study. Those accessions marked with asterisk (*) will be planted in test plots for evaluation when possible.

Code	Accession #	Lab #	Species	Source
*SS	VET-MRL-001	7749	*V. zizanioides*	*cv.* 'Sunshine', Louisiana, USA
*B1	VET-NS-001	8339	*V. zizanioides*	Songkla 1, Thailand
*B2	VET-NS-002	8340	*V. zizanioides*	Surat Thani, Thailand
*B3	VET-NS-003	8341	*V. zizanioides*	Sri Lanka, via Thailand
B4	VET-NS-005	8342	*V. nemoralis*	Kamphaeng Phet, Thailand
B5	VET-NS-006	8343	*V. nemoralis*	Loei, Thailand
B6	VET-NS-007	8344	*V. nemoralis*	Nakon Sawan, Thailand
B7	VET-NS-008	8345	*V. nemoralis*	Prachuap Khiri Khan, Thailand
B8	VET-NS-009	8346	*V. nemoralis*	Ratchaburi, Thailand
B9	VET-NS-010	8347	*V. nemoralis*	Roi Et, Thailand
*ML	VET-TGML-001	8244	*V. zizanioides*	*cv.* 'Malaysia', Malaysia via Spain
*KR	VET-TGKN-003	8246	*V. zizanioides*	*cv.* 'Karnataka', Malaysia via Spain
SH	VET-TGSB-004	8247	*V. zizanioides*	*cv.* 'Sabah', Malaysia via Spain
SB	VET-TGBB-005	8248	*V. zizanioides*	*cv.* 'Sabak Bernam', Malaysia via Spain
PT	VET-TGPB-006	8249	*V. zizanioides*	*cv.* 'Parit Buntar', Malaysia via Spain
*AV	VET-TGAVC-002	8245	*V. zizanioides*	*cv.* 'AVC', Am. Vet. Corp., via Spain
*HF	VET-MB-01	8029	*V. zizanioides*	*cv.* 'Huffman', Florida, USA
*CP	VET-LW-0001	8048	*V. zizanioides*	*cv.* 'Capitol', Louisiana, USA
*SL	VET-RN-001	7951	*V. zizanioides*	Colombo, Sri Lanka
*MA	VET-IMZ-AGA	8349	*V. zizanioides*	Lilongwe, Malawi
*CR	VET-JM-PV1	8076	*V. zizanioides*	Puerto Viejo, Costa Rica
PB	VET-RGG-PA-B	7720	*V. zizanioides*	western site B, Panamá
*ZM	VET-SJC-2	7775	*V. zizanioides*	Zomba, Malawi

RESULTS AND DISCUSSION

The RAPD amplifications resulted in 217 bands. A minimum spanning tree (Figure 1) shows that three major groups are present in the data set: "Sunshine" vetiver, the six putative *Vetiveria nemoralis* from Thailand (B4-B9), and the single Panamá B accession from Panamá. Note that AV (American Vetiver Corp.) and KR (*cv.* Karnataka from Malaysia) show the greatest differences in the "Sunshine" vetiver complex. All of these accessions are non-fertile.

Principal coordinate analysis (PCO) of this similarity matrix removed 69.4% of the variance among the accessions by nine eigenroots. These eigenroots accounted for 20.4, 10.0, 8.2, 6.7, 5.6, 5.2, 4.8, 4.3, and 4.2% of the variance. The eigenroots appear to asymptote after the 5th root. A 3-d ordination reveals that coordinate 1 separated the putative *Vetiveria nemoralis* (Thailand) from all the other accessions (Figure 2). The second axis seems to separate the *V. zizanioides* from Thailand (B1-B3) from other accessions. The Panamá B (PB) accession was separated by the third axis (Figure 2). Clearly, the accessions of *V. nemoralis* from Thailand form a group in both Figures 1 and 2, and this indicates that this group may warrant some taxonomic recognition.

In order to more clearly discern the inter-relationships among the *Vetiveria zizanioides* accessions, the putative *V. nemoralis* accessions and the Panamá B accession were removed from the data set and a new PCO was performed. This PCO resulted in removing 82.1% of the variance among accessions by the first nine eigenroots: 20.0, 12.0, 10.2, 9.3, 7.7, 7.0, 6.0, 5.1, and 4.8%. The first ordination (Figure 3) shows that the "Sunshine" accession occupies a central position with the three Thailand accessions (B1-B3) ordinated away from the main portion of the vetiver accessions. Several Malaysian accessions tended to cluster as a group on the left (Figure 3). The second coordinate shows the unique nature of one of the Malaysia accessions (ML in Figure 3) and the Karnataka (KR) accession. It should be noted that the relationships shown in the minimum spanning network (Figure 1) were unaffected by removing the *V. nemoralis* and Panamá B accessions, because they were not most similar to any of the *V. zizanioides* accessions (data not shown).

Accessions AV (American Vetiver Corp.) and KR (Karnataka, Malaysia) were heavily loaded onto coordinates 4 and 5, respectively. Ordination using axes 1, 4, and 5 reveals that these OTUs are quite distinct (Figure 4). Note particularly that AV is not clustering close to Sunshine (SS), in contrast to Figure 3. This distant relationship to SS is, of course confirmed in the minimum spanning network (Figure

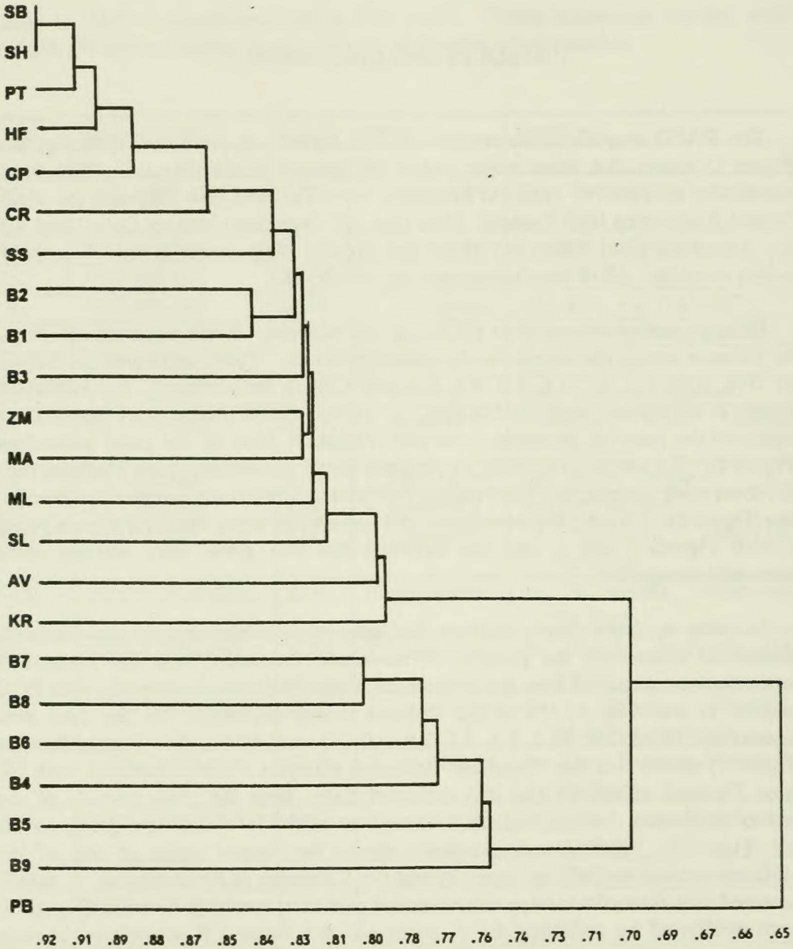

Figure 1. Minimum spanning network for 23 vetiver accessions based on 217 RAPD bands. See Table 1 of code identifications.

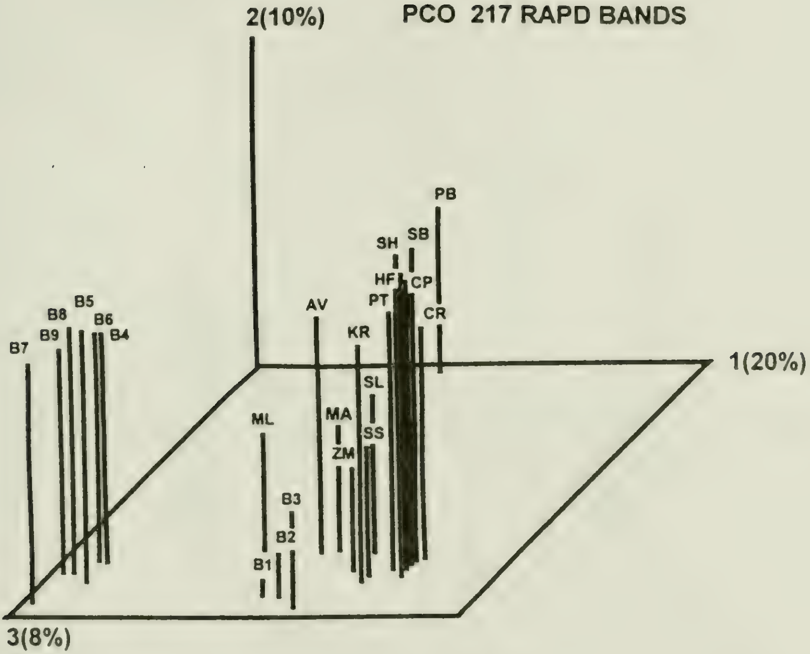

Figure 2. Principal coordinate analysis of the 23 vetiver accessions. Note that accessions B4-B9 are all putative *Vetiveria nemoralis* from Thailand. See text for discussion. See Table 1 of code identifications.

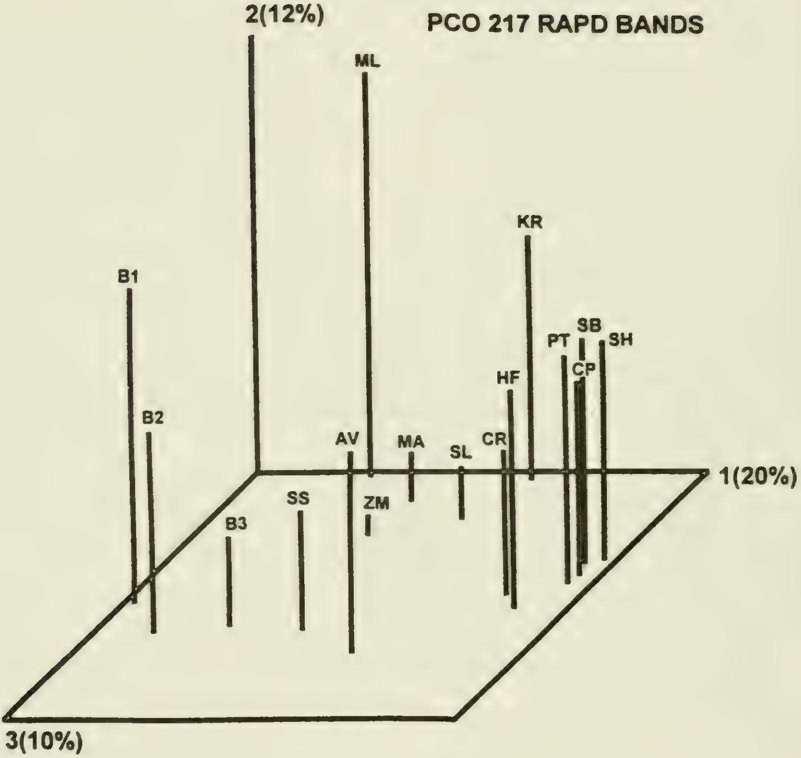

Figure 3. PCO of sixteen, non-seedy vetivers. Note the divergence of the Malaysian accession (ML) and that the Thailand vetivers (B1-3) cluster well with Sunshine (SS). See Table 1 of code identifications

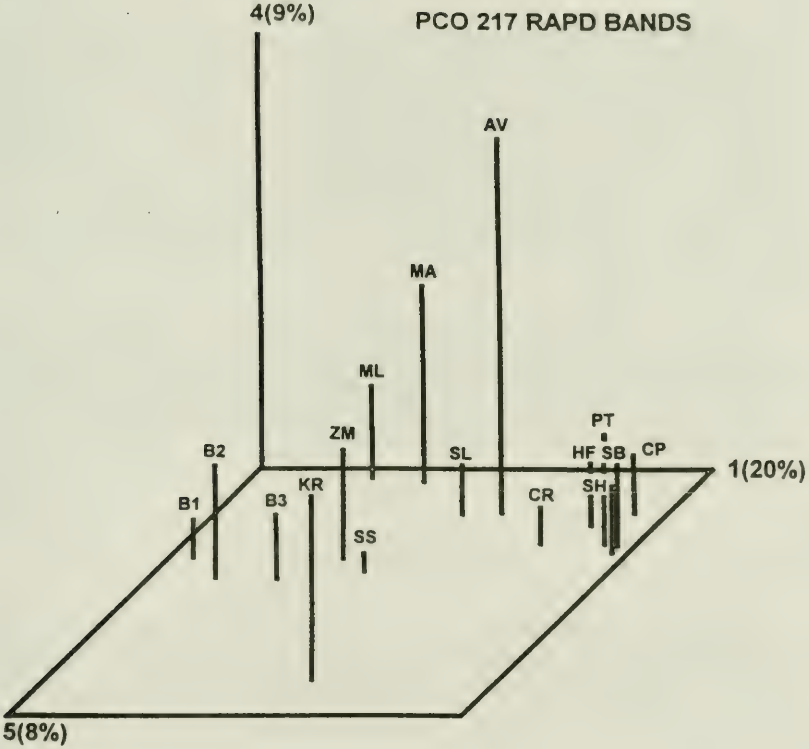

Figure 4. PCO of the sixteen, non-seedy vetivers mapped onto coordinates 1, 4, and 5. Notice the divergence of AV (American Vetiver Corp.) and KR (Karnataka, Malaysia) from the other vetivers. See Table 1 of code identifications

1). It should be noted that there is often distortion when only three axes are used in ordination. In this case, it takes a separate ordination utilizing axes 4 and 5 to portray the variation.

Several points are relevant from this study. New sources of germplasm should be accessioned from the Thailand materials (B1-B3). The Thailand *Vetiveria nemoralis* accessions should be further investigated as to their taxonomic status (species or infraspecific taxon?). In contrast to the previous work (Adams *et al.* 1998), this more robust DNA analysis (217 bands) shows the AV (American Vetiver Corp.) accession to be a source of germplasm that is quite distinct from Sunshine. The Panamá B plants need to be more thoroughly taxonomically investigated. Some of the accessions are so closely related that only one type should be included in test plot evaluation (*e.g.*, SB, SH, PT, HF, see Figures 1, 3), if time and money are constraints.

In order to diversify the current germplasm, we plan to establish test plots in several countries utilizing the following accessions: Sunshine (SS),Songkla (B1), Surat Thani (B2), Sri Lanka (B3) via Thailand, Malaysia (ML), Karnataka (KR), American Vetiver Corp. (AV), Hoffman (HF), Capital (CP), Colombo, Sri Lanka (SL), Costa Rica (CR), and Zomba, Malawi (ZM).

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EUPATORIUM ALBUM L. (COMPOSITAE): NEW TO TEXAS

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ABSTRACT

Eupatorium album L. is reported as new to the state of Texas.

KEY WORDS: Compositae, *Eupatorium*, Texas

Eupatorium album L. is not included in the flora of Texas by Correll & Johnston (1970), nor is it cited by Hatch *et al.* (1990), Johnston (1990), or Jones *et al.* (1996) as occurring in the state. The following collection from east Texas is apparently the first report of the species in Texas.

TEXAS. Anderson Co., Ivy's Preserve, 3 miles WNW of Elkhart, 25 October 1998, *Singhurst & Fleming 7115* (BAYLU).

Ivy's Preserve, located at the western limits of the Pineywoods, is a mature hardwood-pine forest dominated by *Pinus taeda* L., *P. echinata* Mill. (Pinaceae), *Quercus stellata* Wang., *Q. falcata* Michx. (Fagaceae), *Acer rubrum* L. (Aceraceae), and *Liquidambar styraciflua* L. (Hamamelidaceae). Common understory plants include *Rhynchosia latifolia* Nutt. *ex* Torrey & Gray (Leguminosae), *Polygala polygama* L. (Polygalaceae), *Oplismenus hirtellus* (L.) Beauv., *Chasmanthium* *sessiliflorum* (Poir.) Yates (Gramineae), and *Elephantopus carolinianus* Raeusch. (Compositae).

Eupatorium album is distributed in eastern North America from Long Island (NY) to the northern part of Florida, westward to Louisiana, Arkansas, east Tennessee, and the southern counties of Ohio and West Virginia (Gleason & Cronquist 1963; Wunderlin 1998; Strausbaugh & Core 1978). Gandhi & Thomas (1989) report that the species is widespread in the dry woods of Louisiana, which includes two parishes (Sabine and Vernon) that border Texas. Within Arkansas, the species in known in the central and south-central parts of the state (Smith 1988). The Texas record expands the known distribution of the species about 200 km to the west.

In Correll & Johnston (1970), the species would key to *Eupatorium leucolepis* (DC.) Torrey & Gray. The two species may be distinguished by use of the following key, which is adapted from Radford *et al.* (1968), but based upon Texas material.

1. Leaves 5-9 mm wide, the lower surfaces cinereous; phyllaries densely pubescent. . ... *Eupatorium leucolepis*
1. Leaves 10 mm or more wide, the lower surfaces glabrous except for pilose on the larger veins; phyllaries glabrous or pilose only at base............ *Eupatorium album*

The phyllaries of both species are densely covered with globular resinous glands. The glands of *Eupatorium leucolepis* are somewhat obscured by the dense pubescence, while those of *E. album* are much more conspicuous because of the more glabrate nature of the phyllaries. Correll & Johnston (1970) considered *E. leucolepis* to be rare in east and southeast Texas (Hardin, Jasper, Orange, and Tyler counties).

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MIRABILIS MELANOTRICHA (NYCTAGINACEAE), A NEW COMBINATION FOR A COMMON FOUR O'CLOCK FROM SOUTHWESTERN NORTH AMERICA

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ABSTRACT

The new combination ***Mirabilis melanotricha*** (Standley) Spellenberg, based on *Allionia melanotricha* Standley, is proposed for a montane *Mirabilis* common from the southern Rocky Mountains in the United States, through the Sierra Madre Occidental, to the mountains of central México. The plant has been recognized as *Allionia comata* Small, or has been submerged in *Mirabilis oblongifolia* (A. Gray) Heimerl. Taxonomic problems are known to abound in *Mirabilis* L. subgenus *Oxybaphus* Heimerl, probably due to the presence of chasmogamous and cleistogamous flowers and xenogamy and autogamy, combined with an herbaceous perennial habit and phenotypic plasticity. Variation in *Mirabilis melanotricha* is discussed generally, and the species is distinguished from *M. albida* (Walter) Heimerl and *M. oblongifolia*. The name *Allionia comata* is lectotypified.

KEY WORDS: Nyctaginaceae, *Mirabilis*, *Oxybaphus*, *Allionia*, nomenclature

The recent completion of a taxonomic treatment of the Nyctaginaceae for the *Flora del Bajio and Regiones Adyacentes*, under production by the Instituto de Ecologia, Pátzcuaro, Michoacán, México, requires a nomenclatural combination for one of the more easily recognized species of *Mirabilis* in southwestern North America. This combination is also needed for the treatment of the genus now in preparation for the Flora of North America.

Mirabilis melanotricha (Standl.) Spellenb., *comb. nov.* BASIONYM: *Allionia melanotricha* Standl., Contr. U.S. Natl. Herb. 12:351. 1909. *Oxybaphus melanotrichus* (Standl.) Weatherby, Proc. Amer. Acad. Arts 45:425. 1910.

For decades, species in this group of *Mirabilis* were known under the generic name *Allionia*. This name has been conserved for another genus of Nyctaginaceae, the trailing four o'clocks (*A. choisyi* Standl., *A. incarnata* L.), which had been in the genus *Wedelia* Loefl., later *Wedeliella* Cockerell. The species in the traditional *Allionia* were transferred to *Mirabilis* by Standley (1931), but for decades were often considered to belong to the segregate genus *Oxybaphus* L'Her. *ex* Willd. by numerous authors. The use of *Allionia* in this paper refers to described entities for which no name in *Mirabilis* is available.

. *Mirabilis melanotricha* belongs to the subgenus *Oxybaphus* (L'Her. *ex* Willd.) Heimerl, and is part of a complex of forms that has bedeviled students of the genus for nearly a century, not to mention those who have attempted to use their taxonomic treatments. For example, Standley in 1909 and 1911 described numerous entities in *Mirabilis* and segregate genera, only to collapse many into synonymy in his 1918 treatment of the family.

Such was the case with *Allionia melanotricha* and *A. pratensis* Standley, both described at the same time and place by Standley, collected at the same time and same place by J.C. Blumer (TYPES: *A. melanotricha*, Arizona, [Cochise Co.], Chiricahua Mts., Barfoot Park, rolling, andesitic, pine land, recently lumbered, 8000-8250 ft, 17 Sep 1906, *J.C. Blumer 1385* (HOLOTYPE: NMC!; Isotypes: F!,GH!, MO!,NY!,SMU [at BRIT]!,US!); *A. pratensis*, Arizona [Cochise Co.], Chiricahua Mts., Barfoot Park, rolling, andesitic, pine land, recently lumbered, 8000-8250 ft, 17 Sep 1906, *J.C. Blumer 1384* (HOLOTYPE: NMC!; Isotypes: GH!,MO!,NY!,SMU [at BRIT]!). What Blumer saw in the field (two different taxa), Standley put into print, where he noted that *A. pratensis* was obviously related to *A. melanotricha*. Indeed, the two holotypes are remarkably alike, but isotypes of *A. pratensis* are less like *A. melanotricha*. In the original description Standley also stated that according to notes on herbarium specimens, Heimerl had placed the phase that Standley was describing as *A. melanotricha* into a broadly constructed *A. oblongifolia* (A. Gray) Small. In 1911 he noted that *A. melanotricha* was one of the most variable species in the genus, particularly in leaf shape and consistency of blackness of involucral hairs. In 1903 (pp. 407, 1330) Small described *A. comata*, noting that the species had hirsute or hirsute-pilose herbage, the key indicating the stems to be "hirsute," the description saying "hairs viscid, somewhat tangled," and he indicated the species had a geographic range from "Nebraska to Texas and New Mexico." By 1918 Standley had discovered Small's name *A. comata* Small and adopted it, submerging the later-published *A. melanotricha* and *A. pratensis* as synonyms, but indicated the range to be "Arizona and western Texas, southward to Sonora and Puebla."

Allionia comata is typified by a C. Wright collection from southwestern New Mexico (TYPE: *C. Wright 1718*, N. Mex., 1851 (HOLOTYPE: at C. U., not found at NY; Isotypes: GH(2)!,MO!,US!). This collection is actually Wright's field number *314*, "stony hills near the Coppermines, much branching forming large bushes, 20 Aug 1851," located in present day Grant County, near the mining village of Santa Rita. The isotypes are very uniform, to the point of appearing to have come from the same plant. According to Shaw (1987), Gray's distribution number *1718* consists of plants from only one of Wright's field numbers, simplifying lectotypification (LECTOTYPE [here designated]: US #22756). The type is viscid-villous or viscid-pubescent, but not hispid as stated by Small.

In 1931 Standley adopted Heimerl's broad view of a *Mirabilis* with several subgenera and made many numerous nomenclatural transfers from *Allionia* and other segregate genera to *Mirabilis*. He made combinations for those taxa that he currently recognized, such as *M. comata* (Standl.) Standl., leaving synonyms in their respective subgenera. Thus, he never made the new combination from *Allionia* to *Mirabilis* for *A. melanotricha* or *A. pratensis*.

Taxonomy at the species level within the subgenus *Oxybaphus* is problematic. In the introduction to his treatment for *Mirabilis*, Shinners (1951) expressed the kinds of biological and nomenclatural situations that plague classification. Cruden (1973) showed cleistogamic and chasmogamic flowers in *M. nyctaginea* (Michx.) MacMillan and self-compatibility in chasmogamous flowers. The floral mechanism described for *M. nyctaginea* (stamens and style curling together as the flowers wilt after sunrise) is common to chasmogamous flowers of all species of subgenus *Oxybaphus*. It probably insures self-pollination; autogamy is likely. Cleistogamous flowers are found in many other species, including *Allionia pratensis* and *M. oblongifolia*. Nevertheless, chasmogamous flowers in the subgenus are often very attractive and are visited by various strong-flying pollinators, among them various moths, butterflies, bees, and hummingbirds (as is the case in *Mirabilis melanotricha*). Thus, xenogamy is possible. Turner (1993) used the argument of cleistogamy and hybridization, combined with phenotypic plasticity, to support his concept of a widespread and variable *M. albida* (Walter) Heimerl.

Since Standley's time, approaches to the taxonomy of the subgenus *Oxybaphus* either have mostly followed his 1918 treatment or have recognized many fewer taxa. The latter approach was used in the treatment of the genus by Reed (1969) for the Flora of Texas. Reed, working primarily from herbarium specimens and literature, produced a treatment that is an invaluable entrance into the literature and nomenclature of the group, but is often difficult to use in identification of Texas *Mirabilis*. In that treatment he adopted one of Heimerl's names, *M. oblongifolia* (A. Gray) Heimerl for an extraordinarily variable complex of entities from southwestern North America. In that complex, Reed placed *M. comata* as Standley viewed it in 1918 (as *Allionia*). Turner (1993), in providing a taxonomy for the Texas species of *Mirabilis*, recognized even fewer species. He took Reed's *M. oblongifolia* and submerged it and most of its synonyms into an even broader *M. albida*, formerly a comparatively uniform species of the eastern portion of the United States. Turner distinguished his *M. albida* by the presence of a very fine pubescence beneath the larger, more conspicuous, spreading trichomes, a bilayered pubescence that is not consistently present throughout forms of *M. oblongifolia*. Nevertheless, in doing so, Turner noted that *M. comata*, although closely related to *M. albida*, was distinguishable, and he cited a single specimen from extreme western Texas.

From my own study of the type material of *Mirabilis comata*, and from my field work in the vicinity of Santa Rita, N.M., *M. comata*, *M. melanotricha*, and *Allionia pratensis* are different entities and each is recognizable locally. They occupy different habitats: *M. comata* (branched and sprawling, as Wright noted, with semi-succulent grayish, densely viscid-pubescent foliage) from clay-loam soils at mid-elevations in open woodland: *M. melanotricha* (erect, foliage bright green, with thin glabrous leaves) from valleys and slopes at higher elevations usually in meadows and openings in woods; *A. pratensis* (branching at the base with decumbent-ascending branches and rather soft, glabrous, semi-succulent green leaves, the lower of which are often purplish or lead-colored abaxially) from mineral rock in open woodland. With regard

Table 1. Comparison of *Mirabilis albida*, *M. oblongifolia*, and *M. melanotricha*.

	M. albida	*M. oblongifolia* (inc. *M. comata*, *Allionia pratensis*)	*M. melanotricha*
Habit	Usually erect, sometimes decumbent	Erect to decumbent	Erect, rarely decumbent
Leaves	Usually narrow, linear-lanceolate to lanceolate or lance-oblong; usually glabrous; thin to subsucculent; green to grayish green	Usually broadly lanceolate to ovate, more or less triangular, or oval; pubescent or glabrous; thin to subsucculent; green to grayish green	Narrowly lanceolate to ovate or triangular; glabrous; thin; bright green
Inflorescence	Usually with a well-defined main axis and shorter side branches; trichomes of involucre with pale crosswalls	Usually with a main axis and shorter side branches, sometimes rather compact; trichomes of involucre with pale or dark crosswalls (but if leaves glabrous, crosswalls of trichomes in involucre almost always pale)	Appearing more or less evenly forked, without a strong main axis; trichomes of involucre with dark crosswalls
Fruit sculpturing	Ribs high, pale, angular, tuberculate; sulci often with large, bract-like or wart-like tubercles; larger hairs on fruit 0.2-0.3 mm long, tufted	Ribs low, rounded, low-rugose or low-tuberculate; sulci with low rugae or low tubercles; larger hairs on fruit mostly 0.05-0.1 mm, usually evenly distributed	Ribs low, rounded, low-rugose, low-tuberculate, or almost smooth; walls of sulci similarly sculpted; larger hairs on fruit mostly 0.15-0.2 mm long, often tufted
Geographic range	Eastern half of United States and adjacent Canada	Southwestern United States and northern México	Southern Rocky Mountains and Sierra Madre Occidental to central México

to leaves and involucral pubescence, the type of *M. comata* is also very similar to that of *M. oblongifolia*, and for the present the two represent the same taxon. *Allionia pratensis* is more problematic, but also seems to represent a glabrous phase of *M. oblongifolia*.

It is not an exaggeration to say that each species of *Mirabilis* section *Oxybaphus* intergrades with one or more of the other species in the subgenus, such intergradation now explained in some carefully studied floras (*e.g.*, McGregor & Brooks 1986). The mixed breeding system as described by Cruden (1973) for *M. nyctaginea* is apparently widespread in the subgenus. This and the rather high chromosome numbers (the few reported in the literature or on herbarium sheets range between $2n$ = 40 and $2n$ = 58) can be expected to result in numerous populations rather morphologically homogenous within, but varying between, which is clearly apparent in the heterogeneous habitats of south-central North America.

Removing *Mirabilis melanotricha* from the *M. oblongifolia* complex, and not placing the latter into *M. albida*, may make *M. oblongifolia* taxonomically more tractable. For the present, the three taxa may be distinguished in most instances as reviewed in Table 1.

Allionia pratensis and *Mirabilis oblongifolia* often have cleistogamic flowers, particularly on plants where involucres are borne singly in leaf axils. This seems to be an early phenological phase in some individuals; the only phase in others. *Mirabilis melanotricha* is not known to have either of these phases.

Although recognizable by the combination of strictly erect habit, bright green, thin, glabrous foliage, evenly and widely forked inflorescence, dark involucres, and dark cross-walls in involucral trichomes, *Mirabilis melanotricha* is not without its problems. Along the northern edge of its range and at lower elevations in New Mexico it intergrades with *M. linearis* (Pursh) Heimerl through the phase classified as *M. linearis* var. *decipiens* (Standley) S.L. Welsh. In northeastern New Mexico, as judged from the reddish color in fruits of some populations, it may intergrade with the widespread and vegetatively rather similar but more eastern *M. nyctaginea* (Michx.) MacMillan. In the western Texas mountains and occasionally in the Sierra Madre it appears to intergrade with *M. oblongifolia* through the phase similar to a broad-leaved form of *A. pratensis*.

Representative collections of *Mirabilis melanotricha*:

MEXICO. Chihuahua: afueras de Creel (norte), 2400 m., 12 Sep 1988, *R. Mateos 41* (CIIDIR,ENCB); Mcpio. de Zaragosa, Chih. Hwy. 28, 0.5 km S and slightly W of summit of pass through Sierra Catarina, 26 km WSW of Buenaventura, 2290 m, 28 Aug 1996, *R. Spellenberg 12353* (NMC). Distrito Federal: Sierra de Guadalupe, 5 km al NNW de Cuautepec, 2500 m, 29 Jul 1971, *J. Rzedowski 28597* (ENCB,MO). Durango: km 26 de Tepehuanes hacia Guanaceví, 2000 m, 31 Aug 1989, *O. Bravo Bolaños 121* (CIIDIR,MEXU); Mcpio. de Súchil, El Taray, Reserva de Michilía, 2500 m, 28 Jul 1981, *A. Ortega A. 14* (CIIDIR). Guanajuato: 8 km NNW de Santa Rosa por la brecha a Picones, 2340 m, 5 Sep 1998, *S. Zamudio, R.M. Murillo 10830* (IEB). Hidalgo: Mcpio. de Tepeapulco, Ranchería los Cides, 2450 m, 3 Jul 1976, *A. Ventura A. 1722* (ENCB,MEXU,MO,NMC). México, Mcpio. de Huehuetoca, vertiente W del Cerro Sincoque, 2500 m, 3 Aug 1976, *J. Rzedowski 34321* (ENCB,NMC). Puebla: Mcpio. de Libres, San Isidro, 2180 m, 29 Jul 1986, *F. Ventura A. 22232* (ENCB,MEXU). Querétaro: parte alta del cerro Zamorano, 3200 m, 27 Jul 1989, *J. Rzedowski 48781* (IEB).

UNITED STATES. Arizona: Apache Co., Nutrioso Creek, N of Eager, 2290 m, 5 Sep 1942, *R.C. Barneby 5056* (NY); Cochise Co., Chiricahua Mts., Cave Creek Canyon, ca. 5 mi W of Portal, 17 Sep 1988, *R. Spellenberg 9730* (NMC). Colorado: Archuleta Co., 8 mi N of Pagosa Springs, 2400 m, 18 Aug 1936, *R. Rollins 1546* (F, MO,NY). New Mexico: Rio Arriba Co., Chama, 2 Sep 1899, *C.F. Baker 303* (GH, NY); Catron Co., 17.5 mi S of Reserve, 1/4 mi S of Saliz Pass, 1950 m, 29 Sep 1972, *R. Spellenberg 2924* (NMC); Otero Co., E of La Luz in La Luz Canyon, ca. 1 mi W of jxn with Bailey Canyon, S22 T15S R12E, 2 Sep 1987, *R. Spellenberg & N. Zucker 9222* (NMC,NY,TEX). Texas: Culberson Co., Guadalupe Mts. Natl. Park, Bear Canyon, 2320 m, 6 Sep 1987, *L.C. Higgins 17436* (NY).

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FUEL WOOD AS A SOLUTION TO THE ENERGY CRISIS

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ABSTRACT

Wood is the oldest source of useful chemicals, our first source of energy, and the earliest structural and building material. However, as natural forests are depleted in many parts of the world, fast growing trees are becoming more important as a source of fuel wood. A considerable quantity of wood could be easily made available for the production of energy for commercial and industrial purposes by developing forest plantations. A list comprising 25 tree species, which can serve the purpose, is provided here. The species reported here constitute a part of the exhaustive list of tree species that could be recommended as a source of wood for energy production.

KEY WORDS: fuel wood, energy, economic growth

The inevitable transition from traditional to new and renewable energy sources is now taking place in both the developed and developing countries. Solar energy is emerging as a good answer to the challenge. Heavy initial investments and the yet not perfect technology are the problems to be solved. Fuel wood, charcoal, and biomass are the traditional energy sources. Wind, hydro-power and direct solar energy, such as thermal, thermodynamic and photo-voltaic energy, are the renewable energy sources. All of these resources meet only about 15% of the world's current energy needs.

Draught animals, also a source of energy, are widely used worldwide on farms and as a means of transportation. Further research is needed so that this source can be used more efficiently, thereby reducing the current wastage of animal effort. This will increase the working life of the draught animals and may improve the agricultural production. It is important for the developing countries like Pakistan to conserve energy, as high-energy cost is entailed in mechanization. This does not mean that mechanization should be discouraged. If our traditional ways are made more efficient, this will not only conserve our limited energy resources, but also protect our

Table 1. List of tree species which can be used as renewable sources of energy.

Family	Tree species	Common name
Casuarinaceae		
	Casuarina cunninghamiana Miq.	River she-oak
	C. equisetifolia L.	Beaf wood tree
	C. glauca Sieb. *ex* Spreng.	Casuarina
	C. rigidula Miq.	Casuarina
	C. suberosa Otto & Dietr.	Casuarina
Leguminosae		
Mimosoideae	*Acacia modesta* Wall.	Phulai
	Acacia nilotica (L.) Willd. *ex* Del.	Keekar, Gum Arabic tree
	Albizia julibrissin Durazz.	Siris
	Albizia lebbeck (L.) Benth.	Siris
	Albizia procera (Roxb.) Benth.	Black siris
	Albizia saman (Jacq.) Merrill	Monkey pod, rain tree
	Prosopis cineraria (L.) Druce	Jand
	P. juliflora (Swartz) DC.	Mesquite
Papilionoideae	*Dalbergia lanceolaria* L.*f.*	Shisham
	D. latifolia Roxb.	Shisham
	D. sisso Roxb. *ex* DC.	Shisham
	Leucaena leucocephala (Lam.) de Wit	Ipil Ipil
	Robinia pseudoacacia L.	Ainul asal, Honey locust
	Sesbania grandiflora (L.) Pers.	West Indian pea tree
	S. sesban (L.) Merrill	Dhancha, Jantar
Myrtaceae		
	Corymbia citriodora (Hook.) K.D. Hill & L.A.S. Johnson	Lemon-scented gum
	Eucalyptus camaldulensis Delnh.	Red river gum tree
Rhamnaceae		
	Ziziphus jujuba Miller	Berry
Salicaceae		
	Populus × *canadensis* Moench	Poplar
	P. deltoides W. Bartram *ex* Marshall	Cottonwood

Species are arranged alphabetically within genera.

environment. There is a dire need to keep our traditional ways and develop modem technologies side by side as draught animals will still be in vogue in the decade to come.

Wood is the oldest source of useful chemicals, our first source of energy, and the earliest structural and building material (Mahmood & Athar 1997). It is useful to society in providing good quality timber and as a source of a wide variety of derivatives such as resins, gums and oils, and raw materials for the pulp and paper industry (Ilvessalo-Pfaffli 1995). However, as natural forests are depleted in many parts of the world, fast growing trees are becoming more important as sources of fuel wood, construction materials, and good quality timber. One solution to the problem of dwindling forest resources is to provide economic alternatives for those who depend upon the forest for their fuel wood or livelihood (Brown & Lugo 1994). A major alternative is that of creating new forests of fast growing tree species which can provide a range of vital commodities such as fuel, food, fodder, and fertilizer (Athar & Shabbir 1997; Giller & Wilson 1991; Quraishi *et al.* 1993). Increasing the supply of forest products from fast growing tree species will reduce the pressure on native forest resources and contribute to reversing the trend of deforestation. Forests can also be developed in combination with agricultural crops in agro-forestry systems by small scale planting of fuel wood or industrial wood product plantations (Mahmood 1993; Mahmood & Athar 1997; Newman 1997; Subba Rao & Rodriguez-Barrueco 1993). A list of 25 tree species, which can serve the purpose, is presented in Table 1. The nomenclature and taxonomy are after *Index Kewensis*. The list was also discussed with the taxonomists acknowledged in this report. Author citations are quoted following instructions of Brummitt & Powell (1992) as endorsed by the International Working Group on Taxonomy Database for Plant Science (TDWG).

Extensive research is required on fuel wood as a renewable source of energy. Wood production is not only a reliable source of energy; its production also has numerous intangible benefits to the environment. Design of better external combustion engines and turbines using wood as fuel today accounts for 47% of the world's wood consumption. In developing countries, it accounts for about 80% of total wood used as compared to about 10% in developed countries. More than 95% of the households in our rural areas use fuel wood as a primary source of energy, mostly for cooking and domestic heating, and a small proportion for processing agricultural products. The socio-economic condition of rural populations in the developing countries can be improved by streamlining this renewable energy source.

A considerable quantity of wood could be easily made available for the production of energy for commercial and industrial purposes by developing forest plantations. A plantation of fast growing species in plains initially established to meet the domestic fuel requirement could be subsequently converted into an energy plantation for the production of electricity or for the manufacture of liquid or gaseous fuel. A hectare of *Eucalyptus camadulensis* Delnh. can produce up to 21 cubic meters of wood per annum under irrigated conditions in the plains, which is equivalent to 12.1 tons of oven dried wood. A plantation of 10,000 hectares of this species can produce enough wood to feed an electricity generating plant of about 16 megawatts. Such a plantation can also produce about 20,000 tons of ethanol per annum through the process of hydrolysis. Brazil has the largest liquid biofuel production program for motor vehicles in the world (Boddey 1995). The program is based on the production of ethanol (11 to 13 billion L/year) by fermentation and distillation of sugar cane juice, which is used in its hydrated form (93 to 95% ethanol, 5 to 7% water) to fuel 4 million cars, vans, and small trucks. All other light vehicles are fueled with a gasoline and ethanol mix (gasohol), which has a content of anhydrous ethanol that varies from 10 to 22% (Boddey 1995).

Pakistan has a very small wood based industry. Each year, millions of dollars of foreign exchange are spent on importing wood and wood based products. Wood based industries can be encouraged by increasing the supply of locally produced wood. Development of fast growing forest plantations therefore, can result in industrial development in the country without burdening the national energy supply, which is already in severe crisis.

The species reported here constitute a part of the exhaustive list of tree species that could be recommended as a source of wood for energy production. Further research is required for the identification of suitable species for various agro-climatic zones of Pakistan. Because Pakistan lies primarily within arid and semiarid climatic zones (Quraishi *et al.* 1993), research should also be focused in finding better water conservation techniques (Suleman *et al.* 1995). Identification and plantation of drought tolerant and fast growing tree species may be one economically feasible way to increase wood production in water limited environments. The economics of electricity production from wood should be worked out before implementing any project. The study should include the cost analysis of wood production and per unit cost of electrical energy produced.

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OBSERVATIONS ON SOME NEW NODULATING LEGUME SPECIES FROM AZAD KASHMIR

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ABSTRACT

The legumes are well distributed in Pakistan and Kashmir, and Leguminosae ranks as the third largest family in order of abundance. In this paper, a cumulative list of newly reported nodulating legume species from Azad Kashmir is presented. The list comprises eighteen legume species distributed in four tribes: Desmodieae (five species within three genera), Galegeae (nine species within three genera), Indigofereae (one species within one genus), and Phaseoleae (three species within three genera). Observations were also made on the morphological features of the nodules.

KEY WORDS: nodulation, Leguminosae, taxonomy, Azad Kashmir

INTRODUCTION

The role of legumes in reclamation of degraded soils and in the improvement and revegetation of denuded and derelict ecosystems has been well documented (Brockwell *et al.* 1995; Thomas 1995). However, most studies included legumes of agricultural importance, whereas the use of wild legumes has been mainly restricted to the improvement of the productivity of marginal lands. Studies indicate that wild legumes and their associated rhizobia have great potential for increasing or restoring soil fertility (Thomas 1995; Singh & Mahma 1998). The global records of nodulation compiled by Allen & Allen (1981) show that only 15% of legumes have been examined at the species level. However, the percentage of legume species examined has risen to 20% during the last decade with the discovery of more nodulated legume species (Pueppke & Broughton 1999).

The legumes are well distributed in Pakistan and Kashmir, and Leguminosae ranks as the third largest family in order of abundance. Ali & Qaiser (1986) mentioned 107 genera and 539 legume species from Pakistan and Kashmir. Various researchers have made detailed surveys and have tremendously contributed to the studies on nodulation status of legumes of Pakistan and Azad Kashmir (Athar & Mahmood 1978, 1980, 1985, 1990; Nasim & Ahmed 1993; Nasim & Chaudhry 1993; Nasim *et al.* 1994; Mahmood & Iqbal 1994; Athar 1996a, 1997; Athar & Shabbir 1997). In this paper, a cumulative list of newly reported nodulating legume species from Azad Kashmir is presented (Nasim & Ahmed 1993; Nasim & Chaudhry 1993; Nasim *et al.* 1994). Observations are made on the tribal classification of the legume species, and morphological features of the nodules like size, shape, distribution, and color are also described.

MATERIALS AND METHODS

Legumes were surveyed for the presence of nodules from various parts of Azad Kashmir. Both wild and cultivated legumes were examined in their natural habitats at flowering time as described previously (Nasim *et al.* 1994). Legumes examined included weeds, herbs, shrubs, and climbers. At least five plants of each species were examined to minimize error. Nodules were distinguished from other kinds of pathogenic root malformation and many stubby root outgrowths (Truchet *et al.* 1989). In some cases, nodule smears and nodule slices were prepared and examined under the microscope (Somasegaran & Hoben 1994). Nodulation data were recorded and herbarium specimens were prepared for legume identification.

RESULTS AND DISCUSSION

A cumulative list of new nodulating legume species reported by Nasim & Ahmed (1993), Nasim & Chaudhry (1993), and Nasim *et al.* (1994) is presented in Table 1. The nomenclature and tribal classification are as described by Kirkbride (1986). The list comprises eighteen new nodulating legume species distributed in four tribes: Desmodieae (five species within three genera), Galegeae (nine species within three genera), Indigofereae (one species within one genus), and Phaseoleae (three species within three genera). Nodulation is reported in *Atylosa platacarpa* Benth. (Nasim & Ahmed 1993) and in *A. mollis* Benth. (Nasim *et al.* 1994). With the revision in the taxonomy of legumes, *Atylosa* is no longer accepted as a genus and was transferred to *Cajanus*. Nodules were observed for the first time in *A. mollis*, which according to the new classification has been renamed as *Cajanus mollis* (Benth.) van der Maesen.

Nodules were distributed on the main as well as lateral root systems, and were found in the upper 5-10 cm layer of soil. The majority of these species were abundantly nodulated under natural soil conditions, indicating distribution of a wide range of rhizobia in the soil. The shape of the nodules conformed mainly to globose to elongate types (Table 1). However, semi-globose and irregular shapes were also observed. They were usually 2-3 mm in diameter and up to several times longer. The nodules were generally smooth surfaced and occasionally had white fluffy streaks on their surface. These observations corroborate with Corby (1988), Mahmood & Iqbal

(1994), Athar (1996a, 1996b), and Athar & Shabbir (1997) who have described similar morphological features of nodules in various legumes. The size, shape, and color of the nodules varied for various species and sometimes at different stages of development, growth, and maturity. Nodules were mostly pink or brown with reddish interior, indicating their effectiveness in nitrogen fixation (Somasegaran & Hoben 1994).

It has been reported that about 20% of legume species and 57% of legume genera have been examined for nodulation (Pueppke & Broughton 1999). With growing interest in the survey of nodulation both in cultivated and wild legumes, the data would undoubtedly change rapidly in the near future with the discovery of new nodulating species.

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Table 1. Nodule morphology of some new nodulating legume species.

	Plant	Nodule		
Species[a]	Habit[b]	Color	Shape	Frequency[c]
DESMODIEAE				
Campylotropis meeboldii (Schindl.) Schindl.	V	light brown	globose	++
Desmodium motorium (Houtt.) Merr.	S	brown	globose	++
Lespedeza floribunda Bge.	S	brown	globose	++
L. juncea (L.*f.*) Pers. var. *juncea*	S	light brown	globose	+++
L. juncea (L.*f.*) Pers. var. *variegata* (Camb.) Ali	S	light brown	globose	++
GALEGEAE				
Astragalus alopecuroides L.	H	brown	elongated	++
A. chlorostachys Lindley	H	pink	elongated	++
A. hosackioides Benth. *ex* Baker	H	pink	elongated	+++
A. leucocephalus Grah. *ex* Baker	H	brown	elongated	+++
A. psilocentros Fisch.	S	pink	elongated	++
A. subumbellatus Klotzsch	S	light brown	elongated	++
A. trichocarpus Grah.	H	pink	elongated	+++
Gueldenstaedtia verna Georgi	H	brown	globose	++
Oxytropis mollis Royle	H	brown	globose	+
INDIGOFEREAE				
Indigofera heterantha Wall. *ex* Baker var. *gerardiana* (Wall. *ex* Baker) Ali	S	brown	globose	+++
PHASEOLEAE				
Cajanus mollis (Benth.) van der Maesen	V	light brown	elongated	+++
Flemingia fruticulosa Wall.	H	light brown	globose	+++
Rhynchosia pseudocajan Camb.	S	pink	globose	++

[a]: Species are arranged alphabetically within genera. Authors' citations are quoted following instructions of Brummitt & Powell (1992) as endorsed by the International Working Group on Taxonomy Database for Plant Science (TDWG).

[b]: Plant habit: H = herb; S = shrub; V = vine or climber.

[c]: Nodule frequency: + indicates 1 to 5 nodules per plant; ++ indicates 6 to 10 nodules per plant; +++ indicates more than 10 nodules per plant.

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MOLTING HORMONE-LIKE SUBSTANCES FROM *AJUGA IVA* (LABIATAE), ISOLATION AND SPECTRAL IDENTIFICATION

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ABSTRACT

Two phytoecdysteroids were isolated chromatographically from *Ajuga iva* (Labiatae) growing in Jordan. The isolated components were identified as C-29 cyasterone (I); and C-27 ecdysterone (II). The weight percentage recoveries on an air-dry basis of I and II without optimization were found to be 0.03% and 0.02% (w/w), respectively. Structural elucidation of the titled compounds was based on their elemental analysis and spectral data viz. MS, ^{1}H-NMR, IR, and UV.

KEY WORDS: phytoecdysteroids, molting hormones, cyasterone, ecdysterone, *Ajuga iva*, Labiatae

INTRODUCTION

The natural occurrence of bioactive materials in plants warrants a basic research as it suggests the possibility of its exploitation in the medicinal field and/or pest management (Williams 1967; Berkoff 1971; Bowers & Nishida 1980; Saxe 1987; Aranson 1989; Beier 1990). Additionally, the knowledge of the structure of bioactive chemicals makes synthesis of similar or related derivatives quite possible (Jones *et al.* 1986), taking into consideration that synthesis of anthropogenics without guidance is tedious and quite involved.

Ajuga iva is an herbaceous plant grown widely in Jordan, and known there as Ja'da. The extract of this plant is used traditionally as a diuretic, cardiac tonic, or to cure fever and sore throat (Hikino *et al.* 1968; Kubo *et al.* 1982). Several authors (Hikino *et al.* 1968; Kubo *et al.* 1982; Miller *et al.* 1985; Jones *et al.* 1986; Saxe 1987) reported that this plant exhibited antifeedant and insect ecdysis properties, as well as the fact that it has activity against hypercholesterolemia and hyperglyceridemia.

Moreover, it has been reported that *Ajuga* extract exhibits a high stimulating effect on protein synthesis in animals (Robbins *et al.* 1970; Karel 1972).

Reviewing literature on this subject, it was noticed that several bioactive chemicals including clerodane diterpenoid, ajugarines, C-28 ecdysterone, and other ecdysones were isolated from various species of *Ajuga* such as *A. chia* (Poir.) Schreb., *A. iva*, *A. remota* Benth., *A. nipponensis* Makino, and *A. orientalis* L. grown in various parts of the world. However, nothing in the literature could be traced with the phytochemistry of *Ajuga iva* growing in Jordan. Since taxonomy and environmental conditions can play a significant role in variations of plant constituents, it was deemed relevant to investigate this plant as a source of bioactive material that may find a useful application. The search in this field included isolation and spectral identification of the principal constituents of the entire plant.

EXPERIMENTAL

Melting points were not corrected, ^{1}H-NMR spectra were recorded on a Varian HA-100 spectrometer in C_5D_5N. Chemical shifts were expressed in ppm downfield from TMS as an internal standard. Abbreviations: s = singlet; d = doublet. ^{1}H-NMR and microanalysis were carried out by research laboratories at Birmingham University. Electron impact mass spectra were carried out by research laboratories at Nottingham University. IR spectra in KBr were run on a Perkin-Elmer S-21 spectrophotometer. All chemicals and solvents were of HPLC or analytical grade and were used as such.

EXTRACTION AND ISOLATION

The whole fresh plant material was collected locally from north Jordan, identified, washed under running water, air dried in the shade, and ground into small pieces. The entire powdered plant (2 kg) was suspended in methanol and homogenized at ambient temperature according to percolation rules. The combined filtered extract was then concentrated under vacuum and treated with water until 30% (v/v) aqueous methanol was obtained. The final solution was repeatedly extracted with hexane to remove chlorophyll and non-polar coextractants. The mother liquor was then concentrated at reduced pressure to about half its volume and extracted five times with ethyl acetate. The green organic layer was evaporated under vacuum almost to dryness, and finally chromatographed on a silica gel column. Elution was made with $CHCl_3$:MeOH (9:1) with increasing polarity to a ratio of 4:1. Fractions were screened by thin layer chromatography (TLC) in hexane/acetone, and combined together according to their TLC pattern, resulting in the isolation of two crude components; I and II. Yields from the entire plant and without optimization of I and II were 0.03 and 0.02% respectively.

IDENTIFICATION

Crystallization of compound I from MeOH:$CHCl_3$ furnished colorless needles (m.p. 162-164°C), and produced one spot with pink color–positive for steroidal compounds as tested with Sonnenshein's spray reagent on the TLC plate (Karel 1972). Elemental analysis found: C 64.6; H 8.4; O 27.0--required for $C_{29}H_{44}O_8$ (C 64.7; H 8.6; O 26.7). Mass spectrometry produced m/z 520 $M^{+\bullet}$ as a very weak peak relative to the base line at m/z 43. ^{1}H-NMR (Table 1) matches literature values for cyasterone (Hikino *et al.* 1968; Imai *et al.* 1969; Ikan & Ravid 1971a). Acetylation of I in the usual manner (Ac_2O in pyridine at room temperature) gave the respective 2,3,22-tri-acetate, m.p. 251-252°C (elemental analysis found: C 64.7; H 7.5 required for $C_{35}H_{50}O_{11}$ [C 64.99; H 7.8]).

Crystallization of compound II from MeOH:$CHCl_3$ gave a white solid, m.p. 243-245°C and showed one spot with brown color--positive for steroidal compounds as tested with Sonnenshein's spray reagent on the TLC plate. Elemental analysis found: C 67.29; H 9.13; O 23.58--required for $C_{27}H_{44}O_6$ (C 67.47; H 9.23; O 23.30). ^{1}H-NMR (Table 1) matches literature values for ecdysterone (Hikino *et al.* 1968; Imai *et al.* 1969; Ikan & Ravid 1971a, 1971b; Sabri *et al.* 1981; Miller *et al.* 1985).

RESULTS AND DISCUSSION

In the course of our investigation of *Ajuga iva* growing in Jordan as a source of bioactive materials, we found that the crude methanol extract of the whole plant contained two phytoecdysteroids, cyasterone--designated I; and ecdysterone or 20-hydroxyecdysone--designated II (Figure 1). The percentage occurrences of the two chemicals without optimization were estimated as 0.03 and 0.02%, respectively.

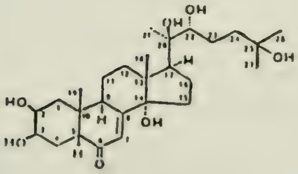
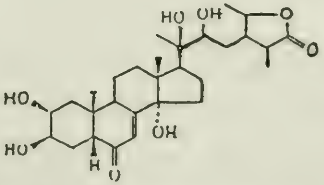


Fig. 1. Cyasteron, I



Ecdysterone, II

Previous studies in this field revealed that various species of *Ajuga* (e.g., *A. chia*, *A. iva*, *A. remota*, and *A. nipponensis*) contain several constituents including clerodane diterpenoid (Camps *et al.* 1982; Jones *et al.* 1986); ajugarins (Shimomura *et al.* 1981; Kubo *et al.* 1982); C-28 ecdysone (Khafagy & Sabri 1979); and steroidal molting hormone-like substances (ecdysones) (Ikan & Ravid 1971a; Sabri *et al.* 1981). In our study, we found that the whole plant of *Ajuga iva* growing in Jordan contains two major ecdysterones; *i.e.*, cyasterone (I), the principal sterol in animals; and ecdysterone (II), the main sterol in plants. In this context, it is worth mentioning that variation among constituents comes as a consequence of species and environmental condition variations.

The structure elucidation of the designated chemicals has been deduced from their elemental analysis and spectral data, as compared with the matched literature values. Elemental analysis of cyasterone was in full agreement with the proposed formula.

The mass spectrum of compound I revealed the correct molecular ion as a very weak peak at m/z (520, $M^{+\bullet}$) relative to the base line at m/z 43. The principal electron impact fragmentations were in good accordance with the assigned structure and were rationalized as follows: peaks at m/z (484, 466, 448, and 430) corresponding to stepwise losses of one to five molecules of water from the parent molecular ion, $[M^{+\bullet}-(1\text{-}5)\ H_2O]$. The peaks at m/z (505, 487, 469, 451, and 433) might be attributed to a fission of one methyl group from the parent ion followed by subsequent losses of one to five molecules of water from $[M\text{-}157]^{+\bullet}$. The rupture of the C_{17}-C_{20} bond followed by hydrogen transfer and loss of water molecules could be the source of the ion at m/z 300. Finally, the fragment at m/z 201 disclosed a C_{17}-C_{20} bond cleavage without rearrangement.

In further support of the suggested cyasterone structure, acetylation of compound I gave the respective 2,3,22-tri-acetate with m.p. and elemental analysis in full agreement with literature values. ^{1}H-NMR spectral data for compound I and its tri-acetate derivative (Table 1), all were in very close similarity to the steroidal nucleus. However, variations occurred in the side chain. It is worth mentioning here that the characteristic bands for acetyl groups on carbons 2, 3, and 22 appeared as singlets at 2, 2.11, and 2.14 ppm. However, protons of carbons 2, 3, 9, 22, and 29 appeared as multiplets down from TMS at 5.02, 5.32, 3.12, 4.08, and 4.1 ppm, respectively.

Table 1. ^{1}H-NMR of *Ajuga* phytoecdysterones in C_5D_5N. Chemical shifts at δ (ppm) from internal TMS. (s = singlet, d = doublet)

	Proton assignments of Carbon:						
Compounds	18	19	21	26	27	29	7
Cyasterone	1.24(s)	1.08(s)	1.57(s)	--	1.34(d)	1.34(d)	6.23(d)
Cyas-triacetate	0.86(s)	1.03(s)	1.25(s)	--	1.30(d)	1.41(d)	5.86(d)
Ecdysterone	1.19(s)	1.05(s)	1.55(s)	1.35(s)	1.35(s)	--	6.2(d)

The characteristic IR bands (cm^{-1}) for compound I at 3480, 1174, and 1618 were assigned for hydroxyl, γ-lactone, and enone systems. The IR bands and the UV absorbing maxima at 243 ($\log\epsilon = 4.1$) together with ^{1}H-NMR singlet at $\delta = 6.23$ ppm, all confirm the proposed C_{29}-cyasterone.

The mass spectrum for compound II did not show the molecular ion. However, the remainder of mass fragmentations together with the m.p., elemental analysis and ^{1}H-NMR data were in full agreement with literature data (Hikino *et al.* 1968; Imai *et al.* 1969; Ikan & Ravid 1971a, 1971b; Mandava 1985; Miller *et al.* 1985) for ecdysterone. The IR and UV spectra were made as a further proof of the assigned structure. ν^{KBr} (cm^{-1}) at 3400, 1650, and 1630 were assigned to hydroxyl, carbonyl, and olefinic groups, respectively. The final IR bands and the UV absorbing maxima at 343 together with the ^{1}H-NMR singlet at δ(6.2 ppm) exhibited by compound II, all confirm the α,β-unsaturated ketone system, and in full accordance with the assigned C_{27}-ecdysterone.

Finally, it is anticipated in this context that a question will arise as to whether the insecticidal potential of the isolated materials was monitored. In reality, the amount dealt with and objectives stated were not geared for this purpose. However, the isolated chemicals are not without precedents. Several authors (Galbraith & Horn 1966; Williams & Salami 1966; Williams 1967; Hikino *et al.* 1968; Ikan & Ravid 1971b; Karel 1972; Mandava 1985) reported that ecdysones and/or molting hormone-like substances from plants mediate several aspects of larval development, its presence over the entire life cycle forces larvae to develop abnormally or fail to mature; and hence it has been suggested that plants synthesize such types of compounds as a self defense against insect attack (Galbraith & Horn 1966; Williams 1967; Mandava 1985), with the advantage that are not only specific but also proof against evolution of resistance (Williams 1967).

In conclusion, two phytoecdysterones, viz. cyasterone and ecdysterone have been isolated and identified from *Ajuga iva* grown in Jordan. In regard to the usefulness, we suggest the possibility of its exploitation in an integrated pest management (IPM) program, provided that the detrimental effect on the environment including man, is assessed prior to its application.

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NOTES ON THE DISTRIBUTION OF *OROBANCHE RAMOSA* L. AND *OROBANCHE UNIFLORA* L. (OROBANCHACEAE) IN TEXAS

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ABSTRACT

The distribution of *Orobanche ramosa* L. and *O. uniflora* L. (Orobanchaceae) in Texas is discussed and mapped.

KEY WORDS: *Orobanche*, Orobanchaceae, Texas, biogeography

Orobanche ramosa L., branched broomrape, is a succulent herb with a yellowish, slender, usually branched stem, approximately 3-15 cm high. Three bracts normally subtend each flower, with the longest one equaling the length of the calyx, which has 4 triangular-ovate, acute, or acuminate teeth. The corolla is 5-9 mm long, has bluish limbs, and a yellowish tube that is slightly constricted above the ovary. The species is commonly parasitic on the roots of tomato, hemp, and tobacco (Britton & Brown 1970).

Orobanche ramosa is a native of southern Europe (Tutin 1964), but has been introduced to the United States, being known from New York and New Jersey to Illinois and Kentucky (Britton & Brown 1970; Fernald 1950) and California (Musselman 1996). The first report of the species in Texas that we have located was by Johnston (1990), which is based upon a specimen that he and K. Nixon collected in Karnes County in 1981. He states that *O. ramosa* is "considered a potentially serious weed pest" and this species has been "the subject of eradication procedures by public agencies." Musselman (1996) mentioned an infestation of *O. ramosa* in Texas discovered in 1982 that was different because the host plants were not crops but roadside weeds. He suggests that the plants were spread by equipment used for roadside mowing. Jones *et al.* (1997) include *O. ramosa* in their checklist and also remark that it is a "federal noxious weed." The species has been the subject of an eradication effort in Texas (Chandler *et al.* 1990).

Specimens of *Orobanche ramosa* were recently collected in the Blackland Prairies of Limestone County, the Post Oak Savannahs of Freestone County, and the Pineywoods of Walker County (see exsiccatae below). In Tutin (1964), the specimens from Limestone and Freestone counties key to *O. ramosa* subsp. *nana* (Reuter) Coutinho, which is distinguished by blue flowers and simple stems. We have not examined the Walker County record. The Texas distribution of this species is depicted in Figure 1.

Specimens cited: TEXAS. Freestone Co.: Donie, corner of Hancock St. and Main St., 8 Apr 1997, *Sammon 51* (BAYLU). Limestone Co.: U.S. Hwy 84 near County Road 120, 3 Apr 1997, *Blackburn 48* (BAYLU,IBE). Walker Co.: Interstate Hwy 45, 1 mi N of intersection with Hwy 30, 5 Mar 1999, *Keith s.n.* (SBSC).

Orobanche uniflora L., one-flowered cancer root or naked broomrape, occurs, according to Correll & Johnston (1970), "In woods and on breaks in isolated localities in e. and w. Tex . . .". Hatch *et al.* (1990) record the occurrence of *O. uniflora* in the Pineywoods, Post Oak Savannahs, Edwards Plateau, High Plains, and Trans-Pecos vegetational regions. We have not located a specimen from the Edwards Plateau, and the High Plains report, which we believe is based on *Correll 23947 & Collins* (LL) from Dickens County, is actually in the Rolling Plains vegetational region. Nesom & Brown (1999) include *O. uniflora* in their checklist of the vascular plants of Walker, Montgomery, and San Jacinto counties, but no voucher specimen is known for any of these three counties. Their report is based upon an unpublished checklist (card file) of the plants of Huntsville State Park, Walker Co., held by the Texas Department of Parks and Wildlife in Austin. The species was collected in the park by Claude A. McLeod, then a professor at Sam Houston State University, and deposited in the herbarium there (SHST). Unfortunately, SHST was totally destroyed by fire in 1978 (*Index Herbariorum*, Pt. 1: *The Herbaria of the World*, 8th ed.).

The following list of exsiccatae documents the known distribution for *Orobanche uniflora* in Texas. The *Truit 87* specimen from Hunt County is notable because it is the first report of the species, which is not included by Diggs *et al.* (1999), in the Blackland Prairies. Additionally, *Gaylord s.n.* from Nacogdoches County, a photographic record, is the easternmost point in the distribution of the species within the state of Texas. Figure 1 presents the distribution of this species in Texas.

Specimens examined: TEXAS. Anderson Co.: 2.3 mi NW of Palestine, 8 Apr 1951, *Shinners 12972* (BRIT). Dickens Co.: on breaks, 1 mi E of Dickens, 28 Apr 1961, *Correll 23497 & Collins* (LL). Hunt Co.: Commerce, Hwy 50, 60 yd from Loop 178, 12 Apr 1995, *Truit 87* (BAYLU). Jeff Davis Co.: rocky open ground near top of Mt. Livermore, 2200 m in Davis Mtns, 14 Jun 1926, *Palmer 30875* (TEX). Nacogdoches Co.: Carizzo Springs branch, Carizzo Springs Drive, 2.8 mi E of Nacogdoches on FM 1878, Mar 1986, *Gaylord s.n.* (BAYLU-photo). Van Zandt Co.: w/o location, May 1930, Thelma Peckham Board School, *leg. ign.* (TEX); 10 mi E of Wills Point, 3 mi E of Edgewood, 16 Apr 1950, *Shinners 12202* (BRIT).

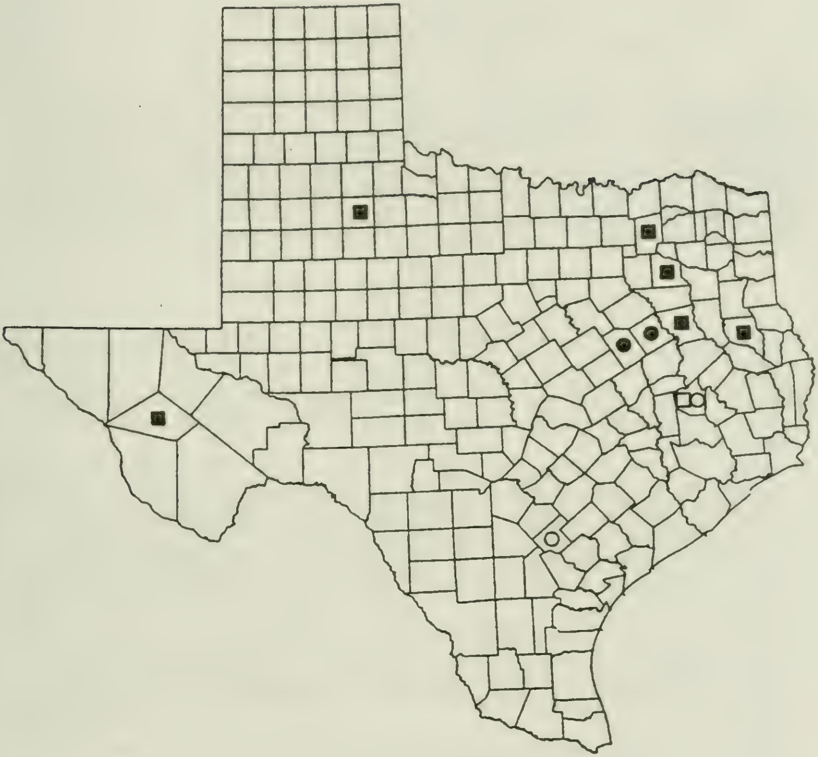

Fig. 1. Distribution of Orobanche ramosa (circles) and O. uniflora (squares) in Texas. Hollow figures represent reports not seen by the authors (see text).

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NEW PLANT RECORDS FOR CENTRAL TEXAS

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ABSTRACT

The distributions of *Chorispora tenella* (Brassicaceae), *Sedum pulchellum* (Crassulaceae), *Rhynchospora nivea* (Cyperaceae), *Hypericum drummondii* (Hypericaceae), *Samolus ebracteatus* var. *cuneatus* (Primulaceae), *Penstemon murrayanus* (Scrophulariaceae), and *Nicotiana repanda* (Solanaceae) in central and north-central Texas are discussed.

KEY WORDS: *Chorispora, Sedum, Rhynchospora, Hypericum, Samolus, Penstemon, Nicotiana*, Brassicaceae, Crassulaceae, Cyperaceae, Hypericaceae, Primulaceae, Scrophulariaceae, Solanaceae, Texas, biogeography

The following series of notes present noteworthy plant records that augment the known distributions for several species that occur in the central and north-central Texas area or, in one instance, discuss another item of importance.

Chorispora tenella (Pall.) DC. (Brassicaceae), a native of Asia, was reported in Texas by Lipscomb (1984) to occur in the following counties: Dallam, Dallas, Deaf Smith, and El Paso. Diggs *et al.* (1999) also list Tarrant County. The specimen of *C. tenella* from McLennan County is noteworthy because it is approximately 175 kilometers south of the nearest record for the species. *Chorispora tenella* is characterized by its purple-blue petals and rather falcate siliques possessing an elongated beak.

Specimen examined: TEXAS. McLennan Co.: FR 1860 at railroad, ca 0.5 mi W of TX Hwy 6, 7.5 mi ESE of Waco, 2 Apr 1996, *Denham 64* (BAYLU).

Sedum pulchellum Michx. (Crassulaceae). The distribution of this species, given by Diggs *et al.* (1999) and largely based upon White *et al.* (1998a), does not include Palo Pinto and Bosque counties, both of which are included in White *et al.* (1998a). While the omission of these two counties could be considered an oversight on the part of Diggs *et al.* (1999), it is equally possible to assume that Diggs *et al.* (1999) consider the report of the species in these two counties to be in error since their manual references White *et al.* (1998a) as the source of the distribution. However, it is clear that neither possibility is correct. The distribution cited by Diggs *et al.* (1999) is based upon the original manuscript submitted to Sida on 5 March 1998 for consideration for publication, which was released to and used by Diggs *et al.* (1999) without the permission or knowledge of the authors of White *et al.* (1998a). The 5 March manuscript did not have the Palo Pinto and Bosque county records because they were not collected until a much later date (see exsiccatae). Both records were added to the revised manuscript, submitted in September 1998, and subsequently published. Thus, the two county records are valid.

Specimens examined: TEXAS. Bosque Co.: intersection of Brazos River and Cedar Creek, 97.32808° N, 31.78761° W, 31 May 1998, *Branch 300* (BAYLU). Palo Pinto Co.: 3.3 mi N of jct Hwy 287 and FM 3027 on Hwy 287, 1.7 mi WSW of Grantham Cemetery, 11 Apr 1998, *Singhurst 6593* (BAYLU).

Rhynchospora (*Dichromena*) *nivea* (Boeck.) Britt. (Cyperaceae) tolerates both sandy and clayey soils along streams, on flats, and in depressions, in the shelter of boulders, thickets and wooded ravines in Arkansas, Oklahoma, and Texas (Correll & Johnston 1970). It occurs on the Edwards Plateau, in south Texas, and adjacent states in México (Correll & Johnston 1970). Diggs *et al.* (1999) list Bell, Brown, Burnet, and Parker counties for the distribution of *R. nivea* within the area treated by their manual, but also state that the species was collected along Turtle Creek, Dallas (Austin Chalk) in 1881 or 1882 by Reverchon. At the time, Reverchon noted that *R. nivea* was "very rare" and it has not been found there since (Thomas 1984; Mahler 1988). The following record for this species is interesting because of two other plants found in the same area: *Tillandsia recurvata* L. (Bromeliaceae; White *et al.* 1998b) and *Samolus ebracteatus* Kunth var. *cuneatus* (Small) Kunth (see below), both of which are unusual for Hill County.

Specimen examined: TEXAS. Hill Co.: wet limestone cliff on E bank of Brazos River below Lake Whitney dam, 97.36468° N, 31.86605° W, 11 Jun 1998, *White 526* (BAYLU).

Hypericum drummondii (Grev. & Hook.) Torrey & Gray (Hypericaceae) is known from Florida to Texas and northward to Maryland, West Virginia, Ohio, Indiana, southern Illinois, Iowa, and southeastern Kansas (Correll & Johnston 1970). Within Texas, *H. drummondii* is found in the central and eastern parts of the state, primarily in dry, sandy, or gravelly soils in abandoned fields, open scrub oak and cedar-oak woods (Correll & Johnston 1970). Hatch *et al.* (1990) indicate the species is known from all vegetational regions within Texas except the Blackland Prairies, High Plains, and Trans-Pecos. Diggs *et al.* (1999) give a rather puzzling distribution for the species in the territory treated by their manual--"in nc TX either in the Cross Timbers or on the sandy soils at the extreme e margin of the area . . .". It is not clear if they are reporting *H. drummondii* in only one or both areas. The following specimens are the first documentation of the species in the Blackland Prairies.

Specimens examined: TEXAS. Falls Co.: Between road and lake on E side of Lake Marlin, 20 Sep 1985, *Gordon s.n.* (BAYLU). McLennan Co.: Abandoned pasture off FM 308, ca 1.5 mi SW of Elm Mott, 24 Oct 1998, *Holmes 9831* (BAYLU).

Samolus ebracteatus Kunth var. *cuneatus* (Small) Kunth (Primulaceae) occurs on wet limestone, in marshes, seepage areas, and in moist soil along streams west from central Texas to New Mexico, south to the Rio Grande Plains, and north to northern Oklahoma (Correll & Correll 1972). Hatch *et al.* (1990) give the distribution for *S. ebracteatus* var. *cuneatus* in Texas as all vegetational regions except the Pineywoods, Post Oak Savannah, and Blackland Prairies. Diggs *et al.* (1999) list Wise and Palo Pinto counties for north-central Texas. The specimens from Bosque and Hill counties, cited below, expand the known distribution for the species in the north-central region of the state.

Specimens examined: TEXAS. Bosque Co.: Cedar Creek at Brazos River, massive limestone cliff, 97.32808° N, 31.78761° W, 11 Jun 1998, *White 531* (BAYLU). Hill Co.: Wet limestone cliff on E bank of Brazos River below Lake Whitney dam, 97.36468° N, 31.86605° W, 11 Jun 1998, *White 520* (BAYLU).

Penstemon murrayanus Hook. (Scrophulariaceae) occurs in sandy soil throughout east Texas, adjacent Choctaw County, Oklahoma; Oauchita and Nevada counties, Arkansas; and Caddo and Winn parishes, Louisiana (Morgan 1995). Thomas & Allen (1998) also report the species as occurring in Natchitoches Parish, Louisiana. Hatch *et al.* (1990) cite the species as occurring in the Edwards Plateau, but Morgan (1995) mentions that the record is probably based upon seeds collected in Wilson County that were cultivated in an agricultural laboratory in Bexar County. For the area treated by their manual, Diggs *et al.* (1999), list Henderson County and Ellis County (also cited by Mahler 1988). The Parker County specimen extends the known distribution of the species about 130 km from its nearest previously known station in Ellis County. This is also the first report of the species in the West Cross Timbers.

Specimen examined: TEXAS. Parker Co.: Peaster, May 1931, *Wiggins s.n.* (TEX).

Nicotiana repanda Willd. *ex* Lehmann (Solanaceae) occurs in southernmost Texas and the adjacent Mexican states of Nuevo León and Tamaulipas (Goodspeed 1954). This species is locally common in creek beds on the Edwards Plateau, but rare in north-central Texas (Correll & Johnston 1970). Mahler (1988) cites a specimen collected by Letterman in Dallas County, but notes that it has "not [been] found there recently." Hatch *et al.* (1990) list the Gulf Prairies and Marshes, Blackland Prairies, South Texas Plains, and Edwards Plateau vegetational regions for this species. *Nicotiana repanda* was recently discovered growing beneath a limestone overhang near the Brazos River in Bosque County (see exsiccata below), which is the first report of the species in the Cross Timbers and Prairies as delineated by Correll & Johnston (1970) and Hatch *et al.* (1990).

Specimen examined: TEXAS. Bosque Co.: Cedar Creek at Brazos River, beneath massive limestone overhang, 97.32808° W, 31.78761° N, 11 Jun 1998, *White 535 (BAYLU).*

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We wish to thank the Herbarium of the University of Texas (TEX), Austin for access to the *Penstemon murrayanus* specimen from Parker County.

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ENEMION BITERNATUM RAF. (RANUNCULACEAE) IN TEXAS

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ABSTRACT

The occurrence of *Enemion biternatum* is documented in Texas. A brief historical account of its mention in literature concerning the state and the plant community in which it occurs is presented.

KEY WORDS: *Enemion*, Ranunculaceae, Texas, biogeography

Enemion biternatum Raf., formerly *Isopyrum biternatum* (Raf.) Torrey & Gray, false rue anemone, is a slender, glabrous perennial herb 10-35 cm tall that has thin fibrous roots and ternately decompound leaves. The flowers are white, disposed in panicles or cymes, with each having 5-6 petaloid sepals. Correll & Johnston's (1970) inclusion of *Enemion biternatum* in the *Manual of the Vascular Plants of Texas*, under the name *Isopyrum biternatum*, was based on a specimen collected in the state by Charles Wright. The species is included in the checklists of Hatch *et al.* (1990) and Jones *et al.* (1997). Ford (1997), in his treatment of the genus for the *Flora of North America*, excludes Texas from the distribution of the species. An inquiry, however, to the Gray Herbarium proved that they indeed have the Wright specimen mentioned above. David E. Boufford relayed the following:

> "We have one specimen of *Enemion* (*Isopyrum*) *biternatum* from Texas, but it may have been overlooked in the past because it is mounted on a sheet [with another specimen] collected by C.S. Short in Kentucky. We have our specimens in alphabetical order by state, say someone looking through quickly may not have noticed that one of the plants was from Texas. Short's label is the more conspicuous of the two. They were both in Asa Gray's herbarium and it was his practice, to save paper I suppose, to mount two or more plants on a sheet. The specimen from Texas is on the right side, but the label in the lower right corner says only 'Herb. A. Gray. *I. biternatum* Torr. & Gray, Fl. N. Am.' and there is a little label on top of that with 'Syn. Fl. N. Am.' The only indication of the locality and collector is a packet in the upper right hand corner with 'Texas, Wright' in Gray's handwriting on the outside. There is no other indication of location."

Correll & Johnston (1970) give the distribution as "possibly in n.e. Texas," which seems based upon proximity to areas in Oklahoma and Arkansas where the species is known to occur. However, we believe the specimen to be from southeast Texas because there is no evidence that Wright visited northeast Texas. Wright came to Texas from Natchez, Mississippi. The most traveled and direct route from Natchez to Texas is via the Harrisonburg Road, an extension of the Natchez Trace, to Natchitoches, Louisiana, then via the El Camino Real (known as the Old San Antonio Road in Texas) to either San Augustine or Nacogdoches, Texas. Geiser (1948), in reviewing Wright's activities in Texas, based upon his (Wright's) correspondence to Asa Gray, states that Wright worked as a surveyor in Angelina, Tyler, Jasper, and Newton counties [all in southeast Texas] from 1837 to 1845. There is no mention in the letters of travel to the northeast part of Texas. Gray received his first letter, now lost according to Geiser (1948), from Wright in 1844. While it is possible that Wright could have traveled to northeast Texas prior to 1844, Gray, in a manuscript autobiography of Wright (apparently now lost, but quoted in Geiser 1948), mentions that Wright made:

> "his headquarters for two or three years [that is, until about 1840, when he went to Town Bluff, a now deserted village in Tyler County] at a place called Zavalla, on the Neches [in present day Jasper County] . . ." [Zavalla is actually in Angelina County.]

The only mention of Wright traveling out of the four mentioned counties concerns a business journey he made during the summer of 1844 to Columbus on the Colorado River, Colorado County. In the spring of 1845, Wright accepted a position with Rutersville College in Lagrange, Fayette County. He never returned to east Texas. Since there is no indication that Wright traveled to northeast Texas, it appears probable that the specimen is from southeast Texas (Angelina, Tyler, Jasper, or Newton counties).

That Correll & Johnston (1970) were correct in expecting the species to occur in northeast Texas is borne out by the following recent Singhurst & Smith specimen from Red River County.

Specimens Cited: TEXAS. Red River Co.: [8.2 miles east of the Jct. of FM 1159 and TX Hwy 37 on FM 1159, then north of FM 1159 ca. 0.9 miles] ca. 1.7 air miles north of Acworth along Greenwood Creek, 16 March 1999, *Singhurst & Smith 7561* (BAYLU, others to be distributed). County and exact location unknown [but probably from Angelina, Tyler, Jasper, or Newton counties), *Wright s.n.* (BAYLU [photo],GH).

In Red River County, the species occurs in the rich humus layer of Roebuck clay soils (Thomas 1977) on relatively flat, moist, and shaded ground adjacent to the base of a steep bluff of 8-15% slope. The hardwood forest present is dominated by *Carya illinoinensis* (Wang.) K. Koch (Juglandaceae), *Quercus macrocarpa* Michx., *Q. muhlenbergii* Engelm., *Q. shumardii* Buckl. (Fagaceae), and *Populus deltoides* Bart. *ex* Marsh (Salicaceae). Subcanopy woody plants include *Sambucus canadensis* L., *Symphoricarpos orbiculatus* Moench. (Caprifoliaceae), *Smilax* spp. (Smilacaceae), and *Cornus drummondii* C.A. Mey (Cornaceae). Common herbaceous species locally associated with *Enemion biternatum* are *Carex* spp. (Cyperaceae) *Erythronium albidum* Nutt., *Polygonatum biflorum* (Walt.) Ell. (Liliaceae), *Impatiens capensis* Meerb. (Balsaminaceae), *Podophyllum peltatum* L. (Berberidaceae), *Parietaria pennsylvanica* Muhl. *ex* Willd. (Urticaceae), *Polygonum virginianum* L. (Polygonaceae), *Ranunculus* sp. (Ranunculaceae), *Sanicula canadensis* L. (Umbelliferae), *Smallanthus uvedalia* (L.) Mack. *ex* Small, and *Verbesina virginica* L. (Compositae).

It is expected that the species occurs in similar situations in southeast Texas.

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NEW NAMES IN THIS ISSUE OF PHYTOLOGIA

As a result of the International Botanical Congress in Tokyo in 1993, the International Association of Plant Taxonomy has been tasked with exploring the feasibility of registration of plant and fungi names. In accordance with terms of the pilot implementation of the registration concept, new names and combinations produced in this issue of PHYTOLOGIA are listed below.

New name or combination	Page Number
Mirabilis melanotricha (Standl.) Spellenb., *comb. nov.*	99

New York Botanical Garden Library
3 5185 00288 4284

Information for Authors

Articles from botanical systematics and ecology, including biographical sketches, critical reviews, and summaries of literature will be considered for publication in PHYTOLOGIA. Manuscripts may be submitted either on computer diskette, or as clean typescript. Diskettes will be returned to authors after action has been taken on the manuscript. Diskettes may be 5.25" or 3.5" and may be written in any IBM or Macintosh compatible format. Typescript manuscripts should be single spaced and will be read into the computer using a scanner. The scanner will read standard type fonts but will not read dot matrix print. Manuscripts submitted in dot matrix print cannot be accepted. Use underscore (not italics) for scientific names. Language of manuscripts may be either English or Spanish. Figures will be reduced to fit within limits of text pages. Therefore, figures should be submitted with internal scales. Legends for figures should be included in figures whenever possible. Each manuscript should have an abstract and key word list. Specimen citations should be consistent throughout the manuscript. Serial titles should be cited with standard abbreviations. References cited only as part of nomenclatural summaries should not appear in Literature Cited. Nomenclatural work should include one paragraph per basionym and must provide proper (as defined by the current *International Code of Botanical Nomenclature*) citation of sources of epithets and combinations.

Authors should arrange for two workers in the appropriate field to review the manuscript before submission. Copies of reviews should be forwarded to the editor with the manuscript. Manuscripts will not be published without review.

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